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TOTAL ORGANISMS PER LITER OF AIR WITH PARTICLE SIZE DISTRIBUTION (TOLA*p*): NEW UNIT OF MEASURE FOR THE TEST AND EVALUATION OF BIODETECTORS

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Detection of biological agents is a complex endeavor, which represents the intersection of the particular detection technology (e.g., PCR, light scatter, immunoassays), the nature of the biological agent (e.g., viral, bacterial, toxin), and the ambient environmental conditions. Therefore, testing and evaluation (T&E) of prototype biodetectors is complicated by the need to consider these variables and to carefully control testing conditions. These considerations can be further complicated depending on the question of application of the data, that is, whether one is conducting a straightforward T&E assessment of competing biodetection technologies to determine their relative capabilities, or using the data to assess health effects and guide subsequent operational decisions. Ideally, a standard unit of measure for a bioaerosol challenge should be applicable to point and standoff detectors and should be able to be translated into operational decisions.

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PREFACE

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TOTAL ORGANISMS PER LITER OF AIR WITH PARTICLE SIZE DISTRIBUTION (TOLA*p*): NEW UNIT OF MEASURE FOR THE TEST AND EVALUATION OF BIODETECTORS

1. BACKGROUND

Detection of biological agents is a complex endeavor, which represents the intersection of the particular detection technology (e.g., PCR, light scatter, immunoassays), the nature of the biological agent (e.g., viral, bacterial, toxin), and the ambient environmental conditions. Therefore, testing and evaluation (T&E) of prototype biodetectors is complicated by the need to consider these variables and to carefully control testing conditions. These considerations can be further complicated, depending on the question of application of the data; that is, whether one is conducting a straight forward T&E assessment of competing biodetection technologies to determine their relative capabilities, or using the data to assess health effects and guide subsequent operational decisions. Ideally, a standard unit of measure for a bioaerosol challenge should be applicable to point and standoff detectors, and should be able to be translated into operational decisions.

2. AGENT CONTAINING PARTICLES PER LITER OF AIR (ACPLA): AN IMPERFECT UNIT OF MEASURE

One historical unit of measure is Agent Containing Particles per Liter of Air (ACPLA). ACPLA can be easily measured for such biological agents as bacterial spores, which are robust enough to survive environmental and collection conditions, and can be cultured using standard laboratory methods. One can calculate the number of particles, which contain agent (i.e., organism or toxin); but, it is impossible to know how many organisms are present, because one particle could contain either one or thousands of organisms. For this reason, two aerosols that both have an ACPLA measure of, for example, 1 may pose vastly different threats (See Figure 1).

The ambiguity in a unit of measure such as ACPLA clearly poses a problem for operational decision making because it provides very little useful information about the total amount of agent present in the environment. From a solely T&E perspective, ACPLA provides a false sense of accuracy and prevents accurate comparisons between detector technologies. For example, suppose there are two competing technologies, Alpha, that can detect as few as 10 organisms, and Beta, that can detect a single organism. In this theoretical example, it is clear that Beta has 10 times the sensitivity of Alpha. However, using a unit of measure such as ACPLA, it is absolutely possible that Alpha and Beta could test identically, or that Alpha could actually test as the *better* technology. Using the same aerosols in Figure 1, Alpha and Beta would register as capable of detecting 1 ACPLA, hence test with equivalent sensitivity, if Alpha is presented with the 1 ACPLA aerosol that actually contains 10 agents. It is important to note that this inaccuracy is not just a theoretical concern. T&E personnel from multiple facilities

informed the Joint Independent Seienee Panel (JISP) that these types of inaeeuraeies do oeeur to some extent during deteetor testing.

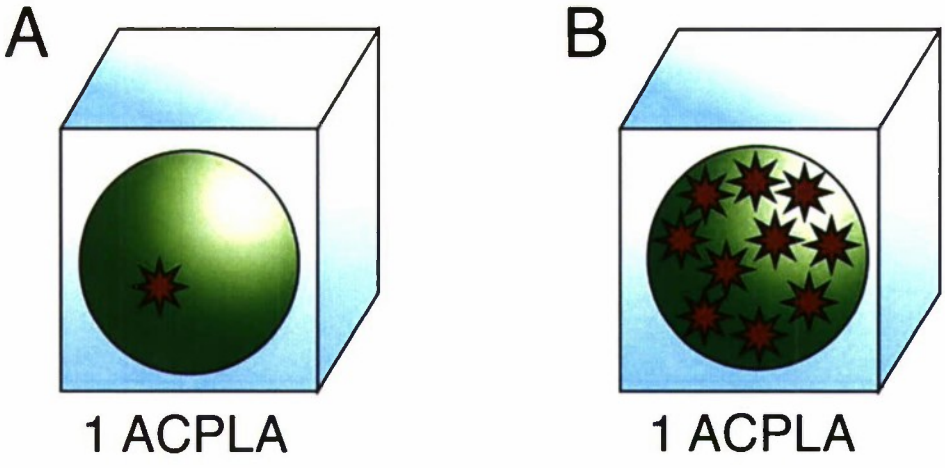

Figure 1. Bioaerosols with the same ACPLA value ean be vastly different.

Both aerosol particles depicted above share an ACPLA value of 1; that is, there is one agent containing particle in the surrounding liter of air. However, the threat posed by the aerosol on the right [B] is substantially greater, as it contains 10 times as many biological agents as the aerosol on the left [A]. ACPLA contains no measurement of the total number of agents present, and hence provides very little information about the aerosols being detected.

Problems with ACPLA are further complieated by the faet that the size of the "agent containing partieles" is not eaptured by the unit of measure. A bioaerosol with particles the size of peas poses a relatively low threat beeause the partieles will settle to the ground quiekly and ean not be readily inhaled. Although this example is obviously extreme, the size of partieles is important beeause partieles beyond 10 mierons in diameter tend to settle rather quiekly and are not effieiently retained in the respiratory traek. This problem is additionally complieated beeause even in the sub 10 mieron range particle size has a very measurable effect on infeetivity and presentation of the disease by determining where in the airway – sinuses, throat, or deep bronehi, for example – the organisms are deposited. Furthermore, many standoff deteetion systems are very sensitive to partiele size. Even if the total number of partieles is the same (1000 per liter of air), a deteetor will reaet differently if those partieles are 1 or 5 microns in diameter. Thus, if a partiele size distribution (PSD) is not speeified within the unit of measure, standoff deteetor teehnologies ean not be properly assessed, eompared, or evaluated. In summary, ACPLA eontains very little useful information that deseribes the bioaerosol, no information about the total amount of agent or whether it is alive or dead, and ean be very diffieult to measure for agents other than baeterial spores.

3. BIOLOGICALLY ACTIVE UNITS PER LITER OF AIR (BAULA): AN INFORMATIVE BUT UNWORKABLE UNIT OF MEASURE

A new unit of measure, Biologically Aetive Units per Liter of Air (BAULA), was reeommcnded by a recent National Research Council (NRC) study. This study also recommended that an additional unit, B*ae*, representing the aerodynamie effieieney of bioaerosol particles, bc added to the calculation. In theory, BAULA and B*ae* together provide a single unit of measurc that is normalized to health effects, and thus is a useful unit with whieh to make operational deeisions. For example, an arbitrary seore of "10" for tularemia would indieate the same health hazard as would a seore of "10" for anthrax, even though the number of pathogens and their PSD differed. Sueh a metrie would address the deficiencies of ACPLA by eonsidering the amount of biologically active agent, the agent's PSD, and the infeetivity of any given biologieal agent.

However, in praetiee, BAULA is virtually impossible to ealeulate for several reasons. First, the infeetivity of most List A biologieal agents is not known, nor is it likely to be known without enormous investments in developing new animal models for these diseases. Second, there is no legitimate way to calculate the viability of the biologieal agent onee it has been distributed as an aerosol. Most agents other than anthrax spores are very labile in the environment and prone to inaetivation as a result of temperature, humidity, ultraviolet radiation, dissemination teehnique, eolleetion technique, and other environmental faetors. Moreover, biodetcetors based on immunological, nucleic acid, or light scattering deteetion cannot diseriminate active from inaetive agent and thus would be incapable of outputting a measurement in BAULA. Third, the effects of PSD, represented by B*ae,* on disposition of biologieal agents within the human target eannot be reliably estimated. Indeed, ealculating health effeets based on different partiele sizes, as BAULA requires, is arguably impossible as sites of deposition, immune response, and breathing rates will likely vary between human and animal models and, indeed, between individuals. In addition, partiele size ealculations are readily aehievable in a pristine laboratory environment, but are extremely difficult in the field where background partieulates make up the majority of the sample. In summary, BAULA is a theoretieally sound, but praetieally insolvable, unit of measure that oversteps the requirements of the T&E process.

4. RATIONAL FOR A NEW UNIT OF MEASURE: TOTAL ORGANISMS PER LITER OF AIR WITH PARTICLE SIZE DISTRIBUTION (TOLA*p*)

With ACPLA insuffieient, and BAULA/B*ae* impraetieal, a new unit of measure is clearly needed for T&E protoeols. From a T&E perspective, viability of the biological agent in an aerosol is not a eoneern beeause the systems being tested detect only presence of an agent and can not discriminate dead from live agent. From the operational perspective, viability of a biologieal agent eould be useful information; however, given that deteetion systems can not determine viability, all commanders will put their troops into a protective posture if any biologieal agent, living or dead, is detected. Therefore, the new measure should not eonsider the viability of the biologieal agent, i.e., live or dead is not an issue. The most important faetor in a unit of measure is an aeeurate representation of the total amount of the biologieal agent present, followed by information on PSD. From a practical and economic point of view, the new unit of

measure should be able to be derived with current technology used in test chambers, ambient breeze tunnels, and field testing.

Key components of a unit of measure for bioaerosol testing and evaluation.

- *Live vs. dead agent is irrelevant since most detector technologies can not discriminate between the two states.*
- *The total number of agents present in a given volume of air is the most important measure of a bioaerosol.*
- *The particle size distribution of a bioaerosol can have a substantial impact on testing results and must be included in a unit of measure.*
- *The unit must be "measurable" in test chambers, ambient breeze tunnels, and in the field, using readily available technologies.*

Herein, we propose a new unit of measure, which combines Total Organisms per Liter of Air (TOLA) with PSD, to be represented as TOLA*p*. The logic for this unit of measure is straightforward. TOLA*p* contains the most important measure of a bioaerosol, the total amount of agent present, and describes the dissemination of that aerosol via the PSD. For T&E purposes, TOLA*p* can be calculated as long as one knows two things: the composition of what is disseminated, and the dynamics of the distribution, including PSD and flow rate. Crucially, both factors are within the control of the tester. The first requirement is to know how many organisms and how much inert material (e.g., filler) are being disseminated. Given a well designed sample preparation protocol, this information should be readily available. The second requirement is to know the PSD. For a solid powder, the PSD is largely dependent on the degree to which the sample has been milled and is readily available. From this point, the dissemination technique has little effect on the PSD. For liquid slurries, the calculation is more complex. Most liquid dissemination techniques are well characterized and generate a fairly consistent PSD. However, because the liquid component of a slurry will rapidly evaporate upon dissemination (leaving only solid components behind), the resulting PSD that is actually presented to a detector will be different. The PSD of this resulting aerosol can still be readily predicted or, in a well designed test chamber, measured directly assuming the composition of the starting material is well characterized. In fact some T&E facilities visited by the JISP have already created models that can make these particle size predictions. From an experimental point of view, it is preferable to measure PSDs directly via a particle sizer, as this method will account for any agglomeration or deagglomeration of particles after dissemination.

From here, it is relatively easy to conceptualize how TOLA would be calculated. Knowing the number of particles being distributed (preferably from direct measurement), the size of these particles (again, preferably from direct measurement), and their composition (from the sample preparation protocol), one can calculate the TOLA. A simplified example is provided in the table. A more formal description of TOLA calculations is provided in the Appendix.

Table. Simplified Coneeptualization of the Caleulation of TOLA.

No. of particles: 5 at 1 mieron, 10 at 5 microns
Sampling rate: 1 liter per second
Sampling Time: 1 second
Total particles per liter of air: 5 at 1 mieron, 10 at 5 microns
No. of organisms per particle: 1 for each 1 micron particle, 125 for each 5 micron particle

TOLA = number of organisms in 1 micron particles + number of organisms in 5 micron particles

TOLA = (1x5) + (10x125) = 1255

The number of particles of differing sizes (1 or 5 microns in this example) can be determined either through direct measurement using a particle sizer or from knowledge of the dissemination technique. From this value, the number of particles per liter of air can be computed using the sampling rate of the particle sizer (chosen as 1 liter per second for simplicity). Knowing the composition of these particles (from the sample preparation protocol), one can determine the number of agents per particle. By combining these values, the Total Organisms per Liter of Air (TOLA) can be calculated.

For the test paradigm to bc relevant, the PSD must be eontrolled and applieable to real life biological warfarc scenarios. For testing purposes, the PSD could bc standardized within the respirable range, generally 1 -10 microns. Ideally, the PSD will match what is mostly likely to be experieneed during a biologieal attaek. Laeking this important pieee of information, deteetors eould be tested at a variety of PSDs (i.e., tests eould be eondueted with 1-5 mieron particlcs and scparately with 5-10 micron partieles). Beyond stating the range of thc PSD, additional information should be reeorded about the "shape" of the distribution. Again, thc PSD "shape" stated in T&E requiremcnts will ideally match the shape likely to be seen in a biological attack. Assuming this information is not forthcoming from thc intelligenee community, a reasonable assumption can bc made that most aerosols will not be monodisperse partieulates. As sueh, some arbitrary distribution shapc may bc necessary (i.e., a normal distribution eentered at x with a standard deviation of y). Multiple well speeified PSDs will be extremely important for testing standoff detectors, whieh may havc vastly different responses, depending on the size of the partieles. See Figure 2.

Onc can readily see that, within the limits of experimental error, the data required to eompute the valuc for TOLAp are available using equipment and proeedures already in placc in a scaled tcst chamber. Morc important, in many cases, one could go back into arehival databascs and perform these calculations on data from previous tests. Granted, PSDs were not speeified or standardized in earlier tests, making direct eomparisons of TOLAp potentially problematic. However, even imperfeet TOLAp comparisons will be more useful than their ACPLA based counterparts.

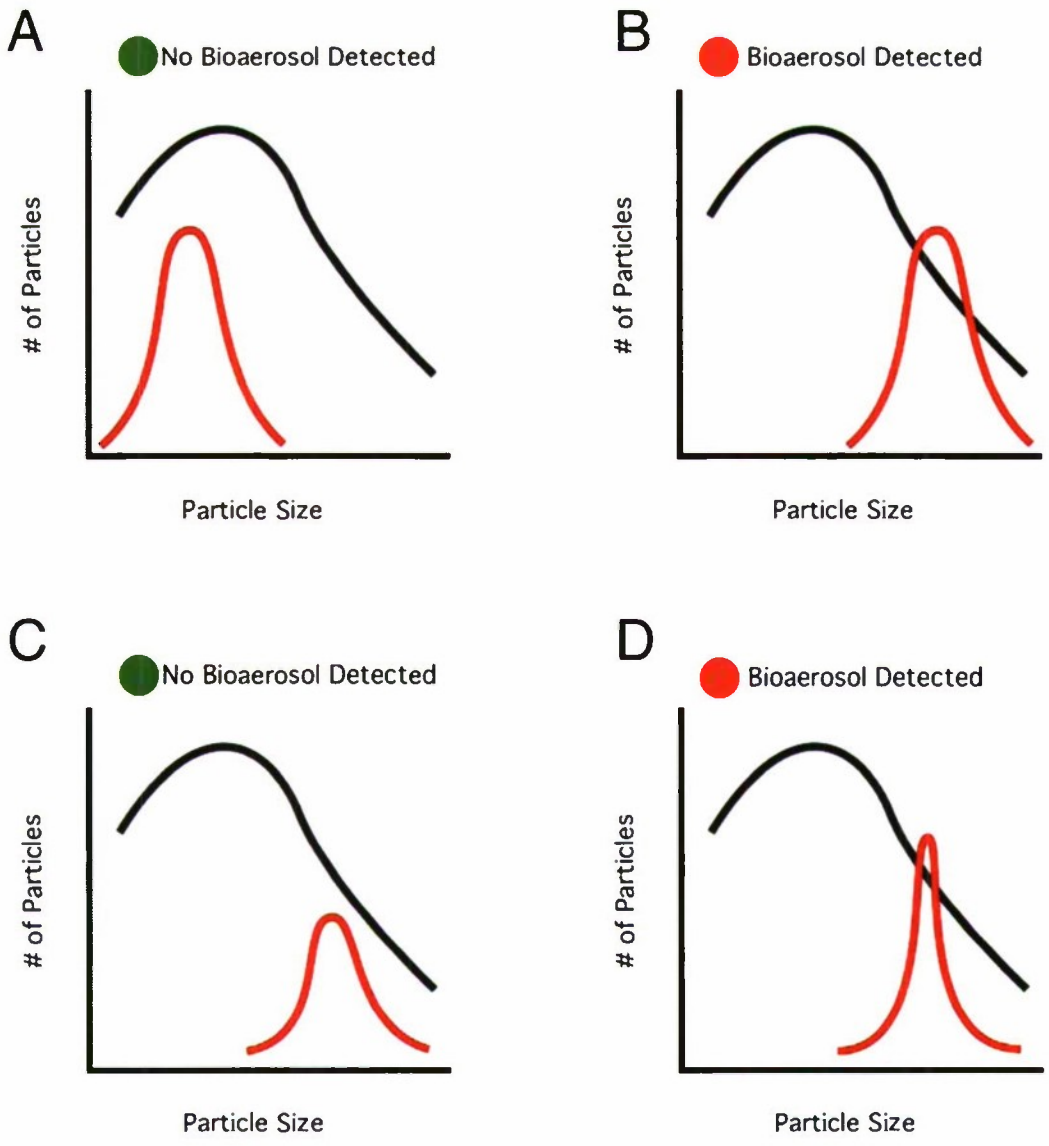

Figure 2. Particle size range and distribution "shape" vastly affect detector testing.

Standoff deteetors have different deteetion thresholds at different partiele sizes (depieted by the blaek lines above). Due to this variable sensitivity, different PSDs that contain an identical number of particles (the red lines) may or may not be detected [A,B]. Beyond the size range, the shape of this distribution ean also affect detector performance as differently shaped distributions, which contain an identical range and number of particles and may or may not cross the detection threshold [C,D].

The progression from chambers to ambient breeze tunnels to large scale open air testing introduces additional variability and experimental error. Although particle sizing and binning is relatively straightforward in a chamber under well controlled conditions, outdoor testing introduces a considerable increase in background particulates (i.e., noise). The cleanest air in the desert around Dugway Proving Ground had background particulates of around 100 ppl; but, this can vary up to 5000 ppl even on a clear day. Some of this background can be readily subtracted from the overall particle count because they will not be in the particle sizes of

relevance to biological warfare (generally 1 to 10 microns). However, some of the background will fall within the 1-10 micron range, emphasizing the need for strict monitoring of the background at all times during the test. In breeze tunnel and field testing, the background particulates may far outnumber those of the bioaerosol such that simple background subtraction may not be possible. One potential technological fix would be to develop simple taggants for test aerosols that would allow particle sizers to discriminate between bioaersol and background particles. Something as simple as green fluorescent protein (GFP) could act as a signal to a fluorescently gated particle sizer, indicating that a particular particle should be counted as part of the bioaerosol. GFP is a standard taggant in biological experiments and industrial scale fermentation processes and could readily be used in the environment without restriction. Notably, fluorescently enabled particle sizers are already commercially available.

5. FINDINGS AND RECOMMENDATIONS

JISP performed an extensive set of interviews with T&E personnel at multiple facilities across the country. By incorporating their comments, concerns, and experiences with the knowledge of the JISP subject matter experts, the JISP reached a consensus on a number of key findings.

a. Key Finding 1: From a T&E standpoint, viability of the organism in a bioaerosol is not a necessary component of a unit of measure.

Current detector technologies cannot differentiate between live and dead biological organisms. Therefore, these technologies are not directly compatible with a unit of measure that makes a distinction between live and dead organisms.

b. Key Finding 2: The most important aspect of a T&E unit of measure is the total number of organisms delivered to either a point or standoff detection system.

The main concern when testing and evaluating a biodetector is to determine its level of sensitivity. The most straightforward and robust way to measure this sensitivity is to determine the minimum number of organisms that can be detected in a given time frame. Notably, the numbers of organisms delivered to the detector are functions of known variables, such as the aerosol composition and the PSD, which are both within the control of the tester.

c. Key Finding 3: Improvements in the generation and dissemination of consistent, well characterized aerosols are necessary. In particular, the ability to present similar aerosols at all scales (test chamber, breeze tunnel, and field tests) is important for rigorous T&E.

To make accurate correlations and extrapolations between different scale tests, aerosols must be generated in a consistent, well characterized measure. Some facilities and T&E personnel indicated to the JISP that improvements were

nccessary in this area. Improvcments in acrosol gcneration at a large scale will be particularly important for standoff detector testing.

d. Key Finding 4: Background particulates can have important effects on detector testing and must be monitored and controlled.

Outside of a testing chamber, background particulates become a concern, particularly as detectors are tested at lower and lower levels of bioaerosol. During T&E of detectors, the background must be closely monitored. In field tests, and perhaps even in breeze tunnels, simulant acrosols could be fluorescently tagged, allowing referee particle sizers and other instruments to accurately count only the particles that are components of the bioacrosol (effectivcly subtracting out background particulates).

e. Key Finding 5: Standardization of testing facilities is not feasible; however, standardization of testing protocols and reagents provides many of the same benefits.

As long as testing chambers and facilities arc well characterized, standardization of these facilities is not required. However, to make accurate comparisons across different test facilities and between detectors, thc production and dissemination of test aerosols must be consistcnt. Several T&E personnel interviewed by thc JISP indicated that the currcnt lack of standardization had led to testing errors.

f. Key Finding 6: Additional research/information is required to accurately translate TOLA*p*, or any unit of measure, into an operation decision regarding health effects.

The main difficulty with thc NRC's recommcnded unit of measure, BAULA, was that it required information such as LD50, amount of live agent, and health impacts of different PSDs. This information is not currently available and may not be available for many ycars to come. To make informed operational decisions, additional research must be conductcd on infectivity of biological agents, likely dissemination techniques (which affect agent viability), and PSDs. Information on dissemination tcchniques and PSDs would serve a dual purposc by allowing more realistic testing of biodctcctors.

g. Key Finding 7: T&E challenge level requirements should include an explicit sampling time component.

Many detector technologics collect samples over various lengths of time. The longer the technology sample collection takes, the higher the sensitivity. Several T&E community members stated concerns that challenge levcl requirements sometimes lacked a sampling time frame, or that the time requirements did not remain constant throughout thc T&E process.

6. FINAL RECOMMENDATION TO THE JOINT T&E OFFICE

JISP recommends that a new unit of measure be introduced for test and evaluation (T&E) of point and standoff biodetectors. This unit of measure, Total Organisms per Liter of Air with PSD TOLA*p*, contains all of the key components necessary to accurately test and evaluate point and standoff detectors. Conveniently, TOLA*p* applies to all types of agents, including spores, vegetative bacteria, and viruses. TOLA*p* can also be applied to toxins by conceptually classifying each molecule of toxin as an "organism." Thus, TOLA*p* can be used as a unit of measure for all agent classes specified by the Joint Test and Evaluation Office (JTEO). JISP recommends that challenge level requirements for biodetectors be stated in TOLA*p* with a given PSD (or several different PSDs) and sampling time. Importantly, TOLA*p* is measureable with current referee equipment, and legacy data should be readily convertible to TOLA*p*.

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APPENDIX

MEASUREMENT OF TOLA*p*

Knowledge of the particle size distribution (PSD) is required to determine the total organisms per liter of air (TOLA); thus, TOLA*p* is readily generated while determining the TOLA. The following calculation assumes a particle counter, which provides the number of particles in a given size range (bin) as a function of particle size, taken here as the radius. This type of particle sizer is readily available and already in use in the bioaersol testing facilities visited by the Joint Independent Science Panel. Although different particle sizers may operate on different principles (e.g., light scattering intensity, aerodynamics, etc.), they all provide the number of particles as a function of size (equivalent radius). If the particles are spherical, then all are essentially the same. Although disseminated biological aerosols (e.g., organisms, filler, surfactants, and/or other extraneous materials) may not always be strictly spherical, this source of error should fall within an acceptable range for T&E (which is an inherently variable process). Because many organisms are not spherical at all, their aggregates in the 1-10 micron size range aerosol will generally be spherical or nearly so.

Two parameters from the particle counter are needed: sampling flow rate (F, L/min) and the sampling time (t, min) used to obtain a given data set. Depending upon the instrument, F may or may not be under the experimenter's control, but will be known. The time t is usually under experimenter control, with larger sampling times leading to more precise PSDs. Depending on the sampling time of the detector, as well as the time course of the entire test, it is likely that a number of separate measurements will be taken during the course of the run. Clearly, multiple data sets can be suitably averaged, so only a single calculation will be used here. The data are normally collected numerically, and are displayed as a histogram in the figure. Here, N_i is the number of particles per liter of air in a bin of size delta R centered at R_i.

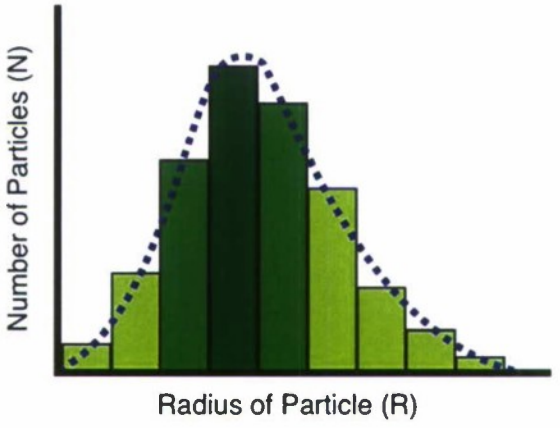

Figure. Hypothetical Output from a Particle Size Counter

Particle sizers "count" the number of particles of various hypothetical radii in various size bins (the green boxes). The various bins can be fit to a distribution curve (blue line), if necessary.

The actual data from the counter is the number of particles in the size bin N_i' (the number of particles collected by the detector in a given sampling period), which can be converted to N_i by dividing N' by Ft ($Ni = N'/(Ft)$). Although Ft is a constant, one could alternatively use N' in the calculation and then divide by Ft at the end.

Now, the total volume of particles contained in 1 liter of air (V) is given by $4/3\pi \sum N_i R_i^3$. (This sum is derived from the equation for the volume of a sphere: $4/3\pi r^3$). If v is the equivalent volume of one organism in the particle (i.e.,volume of the organism, plus any surrounding filler and/or other material), then:

$$TOLA = V/v \qquad (1)$$

The parameter V may be obtained from the composition of the disseminated aerosol, plus knowledge of the average volume of the organism (V_o). For a dry powder, where w is the weight fraction of the organisms in the powder, D is the bulk density of the powder, and D_o is the density of the organism, then $V = (D_o V_o)/(Dw)$. The values of w and D are known from the powder formulation and are under the control of either the experimenter or the tester. The values of D_o and V_o should be available for each organism, and can generally be treated as constants. The values of D_o are expected to be near 1 gram/cubic centimeter, and it may be that this value can be used for all organisms without introducing significant error. If an aqueous slurry is disseminated instead of a dry powder, the value of w to be used in the above expression must be corrected for the weight of water because it will evaporate after dissemination, leaving the dry aerosol. The value is corrected by dividing the weight fraction of agent (organisms) in the slurry by one minus the weight fraction of water in the slurry. To summarize then, in terms of the experimental (measured) parameters,

$$TOLA = (4\pi wD)/(3V_oD_o) \sum N_i R_i^3 \qquad (2)$$

If the data from the particle counter can be approximated by an analytical function (e.g., normal, log normal, Poisson, etc.) as represented by the dotted line in the figure, then the summation can be replaced by an integral.

$$TOLA = (4\pi wD)/(D_oV_o) \int N(R)R^2dR \qquad (3)$$

A note on the "p" in TOLA*p*; clearly, the PSD has been measured, and as stated in the body of this report, can be characterized in a number of ways. If a single parameter is desired, perhaps the best one to use is the volume weighted average radius because it reflects the average size containing the most particles. Finally, it may be noted that with the data collected, it is also possible to weight the distribution for inhalation effectiveness as a function of size if so desired, either now or at some future time.